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Maternal Energy Investment in Eggs and Jelly Coats Surrounding Eggs of the Echinoid *Arbacia punctulata*

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*In free-spawning marine invertebrates, the amount of maternal energy that is invested in each egg has profound implications for all life-history stages of the offspring. The eggs of echinoids are freely spawned into the water and are surrounded by several structurally complex extracellular layers. These extracellular layers, or jelly coats, do not contribute energy to embryonic development but must impose an energy cost on the production of each egg. The investment of maternal energy reserves in the jelly coats of echinoid eggs may have important implications for the number of eggs that can be produced (*i.e., *fecundity) and the amount of energy that can be invested in each egg. We estimated the degree to which maternal energy is invested in the jelly coats surrounding eggs of the echinoid* Arbacia punctulata. *Estimates were derived from measurements of the amount of energy contained in the combined eggs and jelly coats, and in the eggs alone. The amount of energy contained in* A. punctulata *eggs ranged from 2.70 to 5.53* $\times$ 10^{-4} *J egg*$^{-1}$. *The amount of energy contained in the jelly coats ranged from 0.13 to 0.48* $\times$ 10^{-4} *J jelly coat*$^{-1}$. *The mean concentration of energy in the eggs was 2.15 mm*$^{-3}$ *and 0.29 J mm*$^{-3}$ *in the jelly coats. These results indicate that between 3% and 11% (mean = 7%) of the total energy invested in each* A. punctulata *egg is partitioned to the jelly coat alone. A significant positive relationship was found between the volumes of the jelly coats and the amount of energy they contained. Based on this relationship and an analysis of differences in the size of jelly coats between echinoid species, we suggest that the degree to which energy is invested in jelly coats may vary among echinoid species and is therefore likely to be an important life-history characteristic of these organisms.*

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In free-spawning marine invertebrates, the egg contains all the maternal energy provisioned for the development of each offspring. The amount of maternal energy invested in individual eggs is central to many theories on the evolution of life-history patterns in marine invertebrates and is widely considered to have profound implications for all stages of marine invertebrate life cycles (1–5). The eggs of echinoids are freely spawned into the water column where fertilization and development take place. Several extracellular layers surround the eggs of echinoids. These extracellular layers (commonly, and from here on, referred to as "jelly coats") are structurally complex, consisting of several concentric layers of polysaccharide fiber networks embedded in a glycoprotein matrix (6, 7). The jelly coats surrounding the eggs of echinoids are thought to play important roles in fertilization processes (8–10) and may also protect eggs from physical forces that they are exposed to during and after spawning (11, 12). The jelly coats of some echinoid species disintegrate soon after contact with seawater (13) or following fertilization, and do not contribute energy to embryonic development.

Although the jelly coats surrounding the eggs of echinoids do not contribute energy to embryonic development, they must impose an energy cost on the production of each egg. Assuming that the amount of maternal energy available for reproduction is finite, the investment of energy in jelly coats may have important life-history implications. These potential implications include a reduction in the number of eggs that can be produced (*i.e.*, fecundity), a reduction in the amount of energy that can be invested in each egg, or both. Although previous studies have shown that there is substantial extra-embryonic investment in the gelatinous matrices of egg masses that are deposited on the benthos by some marine invertebrates (14, 15), the energy invested in jelly

coats surrounding the eggs of a free-spawning species has not been considered specifically.

We estimated the amount of maternal energy that is invested in the jelly coats surrounding eggs of the echinoid *Arbacia punctulata*. These estimates were derived from wet oxidation (16, 17) measurements of the amount of energy contained in the combined eggs and jelly coats, and in the eggs alone. The wet oxidation method yields an estimate of the amount of organic carbon contained in a sample, which can be directly interpreted as a measure of the amount of energy that it contains. This method has been used in previous studies of maternal energy investment in marine invertebrate eggs (18–21), so our data can be directly compared with earlier results.

The amount of energy contained in the combined egg and jelly coat (mean ± SD = $3.97 \pm 0.79 \times 10^{-4}$ J egg^{-1}) was significantly higher (paired sample t test: $t = 8.33$, df = 9, $P < 0.0001$) than the amount of energy contained in the egg alone (mean ± SD = $3.69 \pm 0.57 \times 10^{-4}$ J egg^{-1}; Table 1). The average (±SD) amount of energy contained in the jelly coat was $0.28 \pm 0.10 \times 10^{-4}$ J jelly coat^{-1}, and constituted 7.4% of the total amount of energy contained in the combined egg and jelly coat (Table 1).

The concentrations of energy (mean joules per cubic millimeter) in the eggs and jelly coats were calculated from the amount of energy each contained (Table 1) and their respective volumes (Table 2). The concentration of energy in eggs was 2.15 J mm^{-3} (*i.e.*, $3.69 \times 10^{-4} \times 5847 = 2.15$ J mm^{-3}, where the combined volumes of 5847 eggs are equivalent to 1 mm^3). The concentration of energy in the jelly coats was 0.29 J mm^{-3} (*i.e.*, $0.28 \times 10^{-4} \times 10{,}416 = 0.29$ J mm^{-3}, where the combined volumes of 10,416 jelly coats are equivalent to 1 mm^3). The concentration of energy in the egg was 7.4 times greater than the concentration of energy in the jelly coats (*i.e.*, concentration of energy in the eggs [2.15 J mm^{-3}] divided by the concentration of energy in the jelly coat [0.29 J mm^{-3}] = 7.4).

Before exposure to seawater, the volume (mean ± SD) of the combined egg and jelly coat was $2.67 \pm 0.30 \times 10^{-4}$ mm^3, and the volume of the egg alone was $1.71 \pm 0.19 \times 10^{-4}$ mm^3 (Table 2). The volume of the jelly coat alone was $0.96 \pm 0.48 \times 10^{-4}$ mm^3 (Table 2); thus, the jelly coat constituted 36% of the volume of the combined egg and jelly coat prior to exposure to seawater (*i.e.*, $0.96/2.67 \times 100 = 35.9\%$). After exposure to seawater, the volume of the jelly coats increased substantially to $9.27 \pm 2.42 \times 10^{-4}$ mm^3 (Table 2) and constituted 84% of the combined volume of the egg and jelly coat.

Linear regression analyses on data contained in Tables 1 and 2 showed no significant relationship between the volumes of the jelly coats and the volumes of the eggs. Similarly, no significant relationship was found between the amount of energy contained in the jelly coats and the amount of energy contained in the eggs. A significant pos-

Table 1

The amount of energy contained (mean ± SD × 10^{-4} J, n = 10) in the combined egg and jelly coat, and in the egg and jelly coat alone for each female Arbacia punctulata; *the proportion of total energy in the combined egg and jelly coat that is partitioned to jelly coat is also given*

Female	Combined egg and jelly coat	Egg	Jelly coat	Energy partitioned to jelly coat (%)
1	4.06 (0.20)	3.93 (0.18)	0.13	3.3
2	3.50 (0.22)	3.15 (0.13)	0.35	10.0
3	5.53 (0.17)	5.32 (0.31)	0.21	3.8
4	4.45 (0.15)	4.12 (0.34)	0.32	7.2
5	4.77 (0.69)	4.29 (0.43)	0.48	10.1
6	3.86 (0.30)	3.59 (0.25)	0.26	6.7
7	3.42 (0.41)	3.05 (0.44)	0.37	10.8
8	2.70 (0.02)	2.47 (0.46)	0.23	8.5
9	3.46 (0.30)	3.11 (0.07)	0.34	9.8
10	4.04 (0.57)	3.89 (0.85)	0.14	3.5
Overall	3.97 (0.79)	3.69 (0.57)	0.28 (0.10)	7.4 (2.8)

Specimens of *Arbacia punctulata* were collected subtidally between July and August 1998 from marina walls at Panama City, Florida. Eggs were obtained from 10 of these specimens by intra-coelomic injection of 0.5–1 ml 0.5 *M* KCl. The amount of energy contained in the combined egg and jelly coat and in the egg alone was determined using a modification of the wet oxidation method given by Parsons *et al.* (16). Energy determinations were made from large samples of eggs that were estimated to yield at least 7.8 joules (J).

The jelly coats were removed from half of the eggs obtained from each female by pouring them through a 100-μm Nytex screen. Thus, samples of eggs with and without jelly coats were obtained from each female for analysis. The concentration of eggs in each sample was determined by replicate counts (n = 7–20) of 10-μl aliquots of well-suspended eggs from each sample. To ensure that the eggs were not damaged by the removal of the jelly coats, eggs were examined microscopically (400× magnification) for any signs of injury to the egg membrane or leakage of yolk from the egg. The viability of eggs from five females was assessed from fertilization assays in which samples of eggs with and without jelly coats were incubated in dilute sperm suspensions (dry sperm diluted by 10^{-4} in seawater). Embryos were allowed to divide to the four-cell stage before being recorded as viable. The proportion of eggs with jelly coats that were fertilized was compared to the proportion of eggs without jelly coats that were fertilized from each female (paired sample t test, $\alpha = 5\%$, on arcsine transformed proportions).

Three subsamples of eggs with jelly coats and without jelly coats were taken from samples of eggs from each female and placed in separate containers. The jelly coat material was eliminated from subsamples by removing the supernatant above the eggs and refilling the container with seawater that had been filtered through a 0.22-μm membrane. This process was repeated several times with all subsamples of eggs. To ensure that all of the jelly coat material had been removed from the subsamples, a vital stain (Janus green) was added to the final supernatant solutions, which were then examined microscopically.

The amount of energy contained in each egg (mean ± SD joules egg^{-1}) was calculated from the total amount of energy in each subsample and the number of eggs that each subsample contained. The concentrations of energy (joules per cubic millimeter) in the eggs and jelly coats were calculated from the amount of energy each contained (Table 1) and their respective volumes (Table 2). A paired sample t test ($\alpha = 5\%$) was used to determine whether there were differences in the amount of energy contained in the combined eggs with jelly coats compared to the amount of energy contained in the egg alone. Relationships between the volumes and the amounts of energy contained in eggs and jelly coats were examined using linear regression analyses. The significance of these relationships were tested by one-way ANOVA ($\alpha = 5\%$).

Table 2

Volumes (mean ± SD × 10 $^{-4}$ mm^3, n = 10) of the combined egg and jelly coat, and of the egg and jelly coat alone before and after contact with seawater, for Arbacia punctulata

	Before contact with seawater			After contact with seawater	
Female	Egg	Egg and jelly coat	Jelly coat	Egg and jelly coat	Jelly coat
1	1.43 (0.22)	3.32 (0.77)	1.89	10.61 (3.08)	9.18
2	2.14 (0.44)	3.49 (0.74)	1.35	11.78 (1.63)	9.64
3	1.73 (0.79)	2.61 (0.35)	0.84	7.45 (1.76)	5.75
4	1.16 (0.31)	2.78 (0.73)	1.62	9.69 (4.04)	8.53
5	1.67 (0.23)	2.56 (0.56)	0.89	15.23 (2.84)	13.56
6	1.73 (0.79)	2.42 (0.31)	0.69	12.77 (1.92)	11.04
7	1.73 (0.79)	2.40 (0.17)	0.67	11.65 (1.60)	9.92
8	1.74 (0.18)	2.29 (0.32)	0.55	12.22 (1.98)	10.42
9	1.70 (0.14)	2.22 (0.25)	0.52	11.15 (1.81)	9.45
10	1.99 (0.52)	2.56 (0.61)	0.57	7.20 (1.19)	5.21
Overall	1.71 (0.19)	2.67 (0.30)	0.96 (0.48)	11.02 (2.51)	9.27 (2.42)

The volumes of the combined eggs and jelly coats and of the eggs alone were calculated from their respective diameters (D) and the equation for the volume of a sphere ($4/3\pi[D/2]^3$). The volumes of the jelly coats were calculated by subtracting the volumes of the eggs alone from the volumes of the combined eggs and jelly coats. Before the eggs of *Arbacia punctulata* contact seawater (*i.e.*, prior to spawning), the jelly coats lie in close proximity to the eggs. After contact with seawater, the jelly coats hydrate and increase substantially in volume. The volumes of jelly coats before hydration were used in calculations of the amount of energy they contain. To determine the pre-hydration volume, the thickness of the coat was measured, using an ocular micrometer in a compound microscope (200× magnification), from the distance between adjacent eggs and added to the mean diameter of the eggs. The edges of jelly coats after exposure to seawater were visualized by adding india ink to the egg suspension, and diameters were measured in the manner described above.

itive relationship was apparent between the amount of energy contained in the jelly coats and their volumes (r^2 = 0.482, F = 7.44, P = 0.025). However, no significant relationship was found between the amount of energy contained in the eggs and the volumes of the eggs.

Microscopic examination of eggs from which the jelly coats had been removed did not reveal any damage to the integrity of the membrane surrounding the eggs. Eggs from which jelly coats had been removed were fertilized at the same rate as eggs with jelly coats at a standard sperm concentration. Thus we assume that the removal of the jelly coats did not result in any leakage of yolk from the eggs or any reduction in their viability.

Our results indicate that approximately 7% (range = 3%–11%) of the maternal energy invested in the combined eggs and egg jelly coats of *A. punctulata* is partitioned to the jelly coats alone (Table 1). The amount of energy contained in the eggs of the *A. punctulata* tested in this study was about half of that reported for this species in a previous study (18). Similarly, the concentration of energy in the eggs of the *A. punctulata* measured here is about half of the average concentration of energy contained in eggs of free-spawning marine invertebrates with planktotrophic larval development (22). Large differences in the amount of energy contained in eggs from different populations of marine invertebrate species have been reported previously (19, 20). Differences in the quality of the yolk content of eggs between populations of the echinoid *Arbacia lixula* have also been reported (23, 24). These population differences in the energy content of the egg and of the quality of the yolk may be the result of variation in the quality and quantity of food available to the adult (22) or of differences in the productivity of the waters in which larvae develop (25).

The degree to which maternal energy is partitioned to the jelly coats of *A. punctulata* eggs (mean = 7.4%) is small relative to the amount of extra-embryonic energy partitioned to the gelatinous matrices of benthic egg masses of some other marine invertebrates. Although these gelatinous matrices contain less energy per unit weight than the eggs they encompass, they constitute a large proportion of the total maternal energy investment in the mass. For example, in species of the prosobranch gastropod genus *Conus*, up to 50% of the maternal energy invested in egg masses is partitioned to the gelatinous matrix (14). Similarly, in species of opisthobranch gastropods, up to 58% of the total energy investment in egg masses is partitioned to the gelatinous matrix (15).

While the amount of energy invested in the jelly coats of *A. punctulata* eggs is small relative to that of the gelatinous matrices of benthic egg masses, it may nonetheless have important life-history implications. Although the jelly coats of echinoid eggs do not contribute energy to embryonic or larval development, they do impose energy costs on the production of each egg. Within the context of current life-history theory (1–5), the investment of energy in the production of jelly coats may influence the number of eggs

Table 3

Size indices of the jelly coats surrounding the eggs of six echinoid species

Echinoids	Source of data*	*n*	Diameter of egg (μm)	Diameter of combined egg and jelly coat (μm)	Relative size index ($\pm$SD)
Strongylocentrotus purpuratus	1	NA	80	120	1.50
Strongylocentrotus franciscanus	1	NA	130	196	1.51
Strongylocentrotus droebachiensis	2	50	160	260	1.61 (0.16)
Arbacia punctulata	3	100	69	126	1.83 (0.15)
Lytechinus variegatus	2	125	143	298	2.09 (0.27)
Dendraster excentricus	4	NA	125	205	1.64

The size indices are the ratio of the diameter of the combined egg and jelly coat (after contact with seawater) to the diameter of the egg alone. A larger index indicates larger jelly coat relative to the size of the egg. SD = standard deviation; NA = not available.

* 1—Lessios, 1990 (25); 2—Bolton and Thomas, unpubl. data; 3—this study; 4—Timko, 1979 (26).

produced, the degree to which energy is invested in individual eggs, or both. Assuming that the maternal energy available for reproduction is finite, and that the amount of energy in each egg is constant, the investment of energy in jelly coats may compromise the number of eggs that could be produced (*i.e.*, may reduce fecundity). This study indicates that approximately 7% of total energy investment in the combined egg and jelly coats is partitioned to the jelly coats alone. Accepting the assumptions given above, the investment of energy in jelly coats may reduce the potential fecundity of *A. punctulata* by about 7%. Alternatively, assuming that the amount of energy available for reproduction is constant, and that the number of eggs produced is also constant, the partitioning of energy to jelly coats may reduce the amount of energy that could be invested in each egg. If this is the case, the investment of energy in jelly coats may compromise offspring growth, survivorship, and reproductive output.

No significant relationships were apparent between the volumes of jelly coats and eggs or the amount of energy contained in jelly coats and eggs. This indicates that the amount of maternal energy invested in jelly coats is independent of the amount of energy invested in eggs. Similarly, no relationship was found between the amount of energy invested in eggs and the volume of the eggs. A significant relationship was apparent, however, between the amount of energy invested in jelly coats and the volume of jelly coats. This suggests that it may be possible to infer the relative degree to which maternal energy is invested in the egg jelly coats of different species from the volumes of these coats.

The proportion of maternal energy invested in jelly coats relative to that invested in eggs is likely to vary among echinoid species. For example, an index of relative size of the jelly coats surrounding eggs of a particular species can be obtained by taking the ratio of the diameter of the egg plus jelly coat to the diameter of the egg alone. Thus, the relative size of the jelly coats to the size of the egg can be compared among species independently of actual differences in egg size. When this index is calculated for the few echinoid species for which data are available (26, 27), differences are apparent (Table 3). Since the amount of energy contained in the jelly coats of *A. punctulata* is positively related to the volume of the jelly coats, it is possible that the proportion of energy invested in the jelly coat relative to that invested in the egg could be inferred from this index. If this is the case, ecologically important differences in the degree to which energy is invested in jelly coats may exist among echinoid species.

The jelly coats surrounding the eggs of echinoids are not unique: the eggs of many free-spawning marine invertebrates are surrounded by extracellular structures and exhibit enormous diversity in size, structure, and form (28–30). Therefore, the investment of energy in the extracellular structures surrounding their eggs may impose substantial reproductive costs on many of these species and should be considered in theories of their life-history evolution. Further measurements of the degree to which maternal energy is invested in the extracellular structures surrounding the eggs of free-spawning marine invertebrates are clearly needed.

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